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To etch or not to etch, Part I: On the fatigue strength and dentin bonding performance of universal adhesives

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ABSTRACT

Objective. To characterize whether the bonding performance and fatigue strength of resin-dentin interfaces created by a universal adhesive would be affected by different H_3PO_4-application times to more accurately assess long-term durability.
Methods. Mid-coronal flat dentin surfaces with standardized smear-layers were produced on sound third molars, etched with 32% H_3PO_4 for 0, 3 and 15 s, bonded with a mild universal adhesive (3M-ESPE) and restored with a nanofilled composite. Bonded specimens (0.9 × 0.9 mm) were stored in deionized water for 24 h and sectioned into beams for microtensile testing (n = 10). Resin-dentin beams were tested under tension until failure (0.5 mm/min) after 24 h or 6 month storage in artificial saliva at 37 °C. Bar-shaped resin-dentin beams (0.9 × 0.9 × 12 mm) were tested under 4-point-flexure initially at quasi-static loads (n = 22) and then under cyclic loads (n > 50). The stress-life fatigue behavior was evaluated using the twin-bonded interface approach by the staircase method at 4 Hz. Fractured interfaces and the tension side of unfractured beams were evaluated under SEM, along with the micro-morphology of the etched dentin surfaces and hybrid layers. Data were analyzed by ANOVA and Tukey test and Wilcoxon Rank Sum Test ($\alpha = 0.05$).
Results. Quasi-static loads were limited to discriminate the bonding performance of resin-dentin interfaces. Application modes significantly affected etching patterns, fatigue strength, endurance limits and hybrid layer morphology ($p < 0.001$).
Significance. Reductions in fatigue strength of self-etched bonded interfaces raise concerns about the true ability of universal adhesives to properly bond to dentin.

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1. Introduction

With the advances in adhesive dentistry [1], strategies to simplify and concomitantly improve resin-dentin interfaces arise as the ultimate aspiration in current product development initiatives. The fairly new class of dental adhesives named as "universal", "multimode" or "multipurpose" has been introduced to allow their use in either self-etch or etch-and-rinse mode according to operator's preference. Universal adhesives are essentially simplified "all-in-one" bonding resins meticulously blended by mixing hydrophobic, hydrophilic and acidic monomers with solvents and initiators within a single bottle. Although added benefits in bonding to glass-rich and -poor ceramics, there is nothing particularly new in this class of adhesives compared to previous generations regarding resin-dentin bonding [2]. The major changes in composition involve the incorporation of silane agents and adequate mixing of acidic monomer-commoner with solvents that solved the bonding mode incompatibility issue. Considering the astounding similarities in composition between universal and the unreliable one-step self-etch adhesives [3], the durability of bonded interfaces produced by such new simplified adhesives remains questionable due to the lack of long-term clinical studies [4–7]. Moreover, establishing which application mode (*i.e.* self-etch *vs.* etch-and-rinse) confers the most favorable dentin bonding performance remains a matter of controversy.

While earlier studies state that universal adhesives in self-etch mode produce better long-term *in vitro* dentin-bonding performance [8–10], recent *in vivo* studies may suggest otherwise [5]. In spite of high-product dependency, the stability of universal adhesives seems to depend largely on their pH [10]. In general, dentin bonding performance of mild universal adhesives (pH $\approx$ 2) tends to outperform ultra-mild (pH $\geq$ 2.5) and intermediately strong (pH $\approx$ 1.5) adhesives [7]. The effect of application mode on dentin bond strengths is claimed to be irrelevant for universal adhesives [6,10–12]. Nonetheless, conventional dentin standardization with 600-grit SiC paper (grit size $\approx$ 14.5 μm) does not necessarily reflect bur-cut dentin surfaces [13]. Regular grit diamond burs (grit size $\approx$ 100 μm) tend to produce rougher-denser smear layers than those of 600-grit SiC (grit size $\approx$ 16 μm) [14]. Curiously, the vast majority of studies employ 600-grit SiC papers for smear layer standardization, which may unwillingly benefit the dentin bonding performance of adhesives used in self-etch mode [13–15]. The assimilation of bond strength results of universal adhesives produced on thin and low-density smear layers may inadvertently mislead material choice and/or the selection of application mode, thus compromising the quality of bonded restorations in the clinical scenario.

The lack of long-term clinical data regarding their performance further complicates clinical decision-making [4–7]. As such, fatigue testing of resin-dentin interfaces emerges as an important clinical predictor to understand and estimate the durability of bonded interfaces in dentistry [16,17]. In mechanical testing of dental restorative interfaces, failures associated with overloads are generally considered first. However, fatigue is more often the primary mode of fracture of load-bearing structures [18]. The most important characteristic of fatigue is that failures occur at stresses that are generally lower than conventional quasi-static loading strength testing due to the growth and coalescence of intrinsic flaws within the materials [16]. Surface integrity, characterized by flaw sizes and distribution, plays an important role on the fatigue strength of bonded interfaces [16–18]. Hence, flaws introduced during the bonding/restorative stage (*e.g.* dentin-etching and surface preparation), may affect fatigue strength and compromise long-term durability [19] to a greater extensions than in conventional bond strength testing. Characterization of the stress-life fatigue behavior is a powerful, albeit underused approach to determine the endurance limit of bonded interfaces in adhesive dentistry. Understanding the differences involved in the self-etch and etch-and-rinse bonding mechanisms of universal adhesives by means of a more discriminative fatigue-testing approach could help elucidate inconsistencies regarding the indication of dentin-etching for universal adhesives. Therefore, the primary aim of this study was to compare the discriminative ability between a fatigue testing approach and conventional quasi-static loading to better understand the effect of H_3PO_4 application times on resin-dentin bonding. The notion that conventional quasi-static loading microtensile testing provides clinically relevant insights on dentin bond durability was challenged by a modified fatigue-testing setup composed of comparable specimen sizes. The objective was to assess the effect of application modes including different H_3PO_4 application times on the resin-dentin bonding performance of a universal adhesive. The null hypotheses tested were that: *(i)* dentin-etching with phosphoric acid would have no impact on the dentin bonding performance of the tested mild universal adhesive and *(ii)* the ability to identify differences in bond strength performance would not be affected by the test method (*i.e.* quasi-static loading methods and the fatigue test).

2. Materials and methods

Seventy-four extracted sound human third molars were obtained with informed consent from patients (age 18–30 years) under a protocol (#23-2003) approved by the University of Oulu, Finland. Teeth were stored at 4 °C in 0.9% NaCl containing 0.02% NaN_3 to prevent microbial growth and were used within 1 month after extraction.

2.1. *Experimental design and bonding protocols*

The durability of resin-dentin bonds resulting from a mild universal adhesive (Scotchbond Universal Adhesive: SU, 3M ESPE; pH 2.7) [7,20] used in self-etch and two etch-and-rinse modes was assessed in terms of 4-point flexural strength (n = 22/group), stress life fatigue (n > 50/group) and microtensile bond strength (n = 10/group). Hybrid layer and etched-dentin morphology were evaluated by SEM. The experimental design was composed of one study factor: dentin-etching depth, in three levels composed of self-etch application, 15 s and 3 s etching. Specimen preparation followed the Academy of Dental Materials guidance of *in vitro* testing for non-trimmed microtensile bond strength testing [21] and flexure strength and stress-life fatigue behavior were evaluated using the twin-bonded interface (TBI) approach [17], with the exception that cross-sectional area of specimens was reduced to 0.82 mm^2 (±0.15). Smaller cross-sectional areas were deliberately selected for the fatigue setup to allow a more realistic comparison between 4-point flexural and the commonly used microtensile static tests. Standardized smear layers were produced by wet-polishing the dentin surfaces with 320-grit SiC paper (Buehler-MET II, Buehler; grit size ≈ 36 μm), for 30 s. After dentin etching for 3 or 15 s with 32% H_3PO_4 (wt%) (Scotchbond Universal Etchant, 3M ESPE), dentin surfaces were rinsed for 15 s, blot-dried with lint-free absorbent paper following the wet-bonding technique [22] and one coat of the universal adhesive was actively applied on the dentin surface under manual pressure equivalent to approximately 40.5 g (±9.3) for 20 s. Solvent evaporation and adhesive thinning were gently performed for 10 s, followed by light-curing for 10 s using a LED unit (Elipar Deepcure, 3M ESPE) at 1400 mW/cm^2. For the self-etch mode, a similar protocol was employed with the exception that dentin-etching was not performed and the dentin surface was air-dried for 5 s, but not overdried before bonding according to the manufacturer's instructions. A nanofilled composite (Filtek Supreme XTE, 3M ESPE, St. Paul, MN, USA) was used as the restorative material. All bonding procedures were carried out by the same pair of calibrated operators.

2.2. *Characterization of the fatigue behavior*

Bar-shaped dentin beams, roughly 1 mm × 1 mm × 8 mm, were obtained from 28 sound third molars. Roots were removed 1 mm below the cervical line and discarded. Crown segments were longitudinally sectioned occluso-cervically to produce mesio-distal slabs which were wet-polished with 320-grit SiC paper (Buehler-MET II, Buehler) for 30 s and perpendicularly sectioned at the mid-coronal region with a slow-speed diamond saw (Isomet, Buehler Ltd, Lake Bluff, IL, USA) under water cooling. Dentin beams were randomly selected and the twin-bonded interface specimens (TBI) were produced using a molding process described by Mutluay et al., [17]. Briefly, bonding was concomitantly performed on opposing dentin surfaces and the beams were placed inside a dedicated mold with the tubules oriented nominally parallel to the bonding interface. The restorative composite (Filtek Supreme XTE, 3M ESPE) was applied in a single increment to fill the mold cavities on both sides of the dentin beam. The composite was cured for 20 s on both sides using a LED unit (Elipar Deepcure, 3M ESPE) with output intensity of 1400 mW/cm^2 and with tip diameter wider than 10 mm. The bonded sections were released from the mold, inspected for voids and flaws, lightly wet-polished with 600- and 1200-grit SiC paper and sectioned with a slow-speed diamond saw (Isomet, Buehler Ltd) to obtain twin-bonded interface samples roughly 0.9 mm × 0.9 mm × 12 mm. A minimum of 50 TBI samples were prepared for each group (n > 50/group) with average cross-sectional area of 0.84 mm^2 (±0.15). Specimens were re-inspected for flaws at the bonded interface using a stereomicroscope (Leica M60, Leica Microsystems) with 40× magnification and stored in artificial saliva [23] at 37 °C for a minimum of 48 h prior to further evaluation. TBI specimens were evaluated under quasi-static and cyclic four-point flexure using a universal testing system

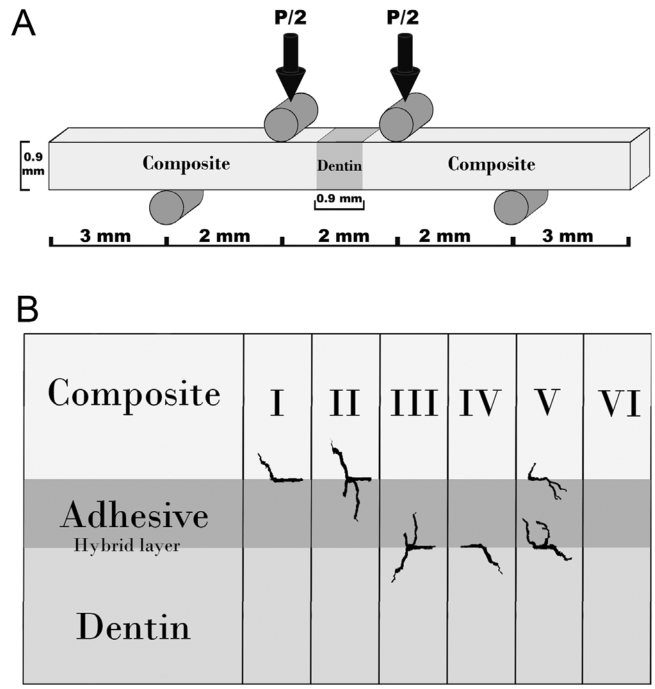

Fig. 1 – Schematic diagram of specimen configuration and loading arrangements used for characterizing the 4-point-flexure (n = 22) at quasi-static loads and stress-life fatigue behavior at 4 Hz (n > 50) at cyclic loads of resin-dentin interfaces bonded with a mild universal adhesive.

(Electropuls E1000, Instron) with load capacity of 250 N and sensitivity of 0.025%. All experiments were performed with specimens fully immersed in artificial saliva at room temperature. TBI specimens were placed on a fixture so that the load was applied on the occlusal surface (Fig. 1A). Quasi-static loading was applied at a rate of 0.05 mm/min. The flexural strength (FS) of the beams was calculated using conventional beam theory [24] in terms of the maximum measured load (P) in N and beam geometry (width b, thickness h in mm) according to $FS = 3\ Pl/bh^2$, where l is the distance from interior and exterior supports (l = 2 mm). Twenty-two specimens (n = 22) were evaluated per group. Cyclic loading of the TBI specimens was conducted using the same flexure configuration under load control with frequency of 4 Hz and stress ratio (R = ratio of minimum to maximum cyclic load) of 0.1. The cyclic loading experiments followed the staircase fatigue method and beginning at approximately 90% of the flexural strength, identified from the quasi-static loading, followed by sequential reductions in the order of 10% until failure. The process continued until reaching a flexure stress amplitude (MPa) at which the specimens did not fail within 1.2×10^6 cycles. The cyclic stress amplitude was plotted in terms of the number of cycles to failure in log-base format. The data was fit through a non-linear regression with a Basquin-type model, according to equation $\sigma = A(N)^B$, where A and B are the fatigue-life coefficient and fatigue-life coefficient exponent, respectively. The apparent endurance limit was estimated from the models for a fatigue limit defined at 1×10^7 cycles [17,25]. A minimum of 50 TBI specimens were evaluate per group.

2.3. *Detection of crack initiation sites*

The unfractured sides of TBI specimens which withstood 10^4 loading cycles or more were evaluated by scanning electron microscopy (SEM) to identify the origins of failure and potential weak links at the bonded interface. Specimens were lightly wet-polished with SiC papers 600-, 1200-, 2000- and 4000-grit SiC paper followed by dehydration in a series of ascending ethanol series (50, 70, 80, 90 and 3 × 100%), fixed in hexamethyldisilazane, sputtered with gold/palladium and analyzed on backscattering mode at 10 kV (Phenom ProX, Phenom-World). SEM micrographs (5000× magnification) were taken sequentially covering the entire extension of the bonded interface locate at the tensile side of the specimens. Crack initiation sites were classified by a blinded-calibrated operator into 6 categories according to crack locations: (I) between composite and the bonding resin or cohesively at the surrounding composite; (II) between composite and adhesive layer extending towards the bulk of the adhesive layer; (III) between dentin and adhesive layer extending towards de bulk of the adhesive layer; (IV) between dentin and the adhesive layer or cohesively at the surrounding dentin; (V) mixture of type II and III; and (VI) no cracks were identified (Fig. 1B).

2.4. *Microtensile bond strength (μTBS) testing*

Thirty sound third molars (n = 10/group) were sectioned under water cooling to expose flat dentin surfaces using a slow speed diamond saw (Isomet, Buehler Ltd., Lake Bluff, IL, USA). Absence of remaining enamel on the dentin surfaces was verified with a stereomicroscope (Leica M60, Leica Microsystems) at 40× magnification. Roots were removed 1 mm below the cervical line and discarded. Exposed midcoronal dentin surfaces were wet-polished with 320-grit SiC paper for 30 s. Crown segments were randomly allocated to 3 groups following the same bonding protocols used for quasi-static flexure strength and stress-life fatigue behavior. Composite blocks were built with a nanofilled composite resin (Filtek Supreme XTE, 3 M ESPE) in 2 increments of 2 mm. Each increment was light-cured for 40 s (Elipar Deepcure, 3M ESPE). All bonding procedures were carried out by the same pair of calibrated operators. The restored crown segments were stored in distilled water for 24 h at 37 °C and resin–dentin beams were produced with a cross sectional area of 0.88 mm^2 ± 0.19 by sectioning the restored crowns longitudinally in mesio-distal and buccal-lingual directions perpendicular to the bonded interface with a slow-speed diamond saw (Isomet, Buehler Ltd.). A minimum of 18 resin-dentin beams were produced per tooth.

Resin-dentin beams were then randomly selected to be tested under two conditions: immediate testing after 24 h of storage in distilled water at 37 °C and long-term aging after 6 months at 37 °C in artificial saliva composed of 5 mM HEPES, 2.5 m M $CaCl_2{\cdot}H_2O$, 0.05 mM $ZnCl_2$, and 0.3 mM NaN_3, pH 7.4 [23], which was changed biweekly to prevent pH changes. In order to obtain a research design balanced by tooth dependency [21], beams from the same tooth were submitted to each of two testing periods: 24 h and 6 months. A minimum of 7 beams per tooth (n = 10) were tested on each storage period. Beams were tested under tension on a mechanical testing machine (Bisco, Schaumburg, IL, USA) at a crosshead speed of 0.5 mm/min until failure to obtain the maximum load *(P)* in N. The cross-sectional area *(CA)* in mm^2 of each beam was measured with a digital caliper to nearest 0.01 mm. The formula $\mu TBS = P/CA$ was used to calculate μTBS values in MPa. Tooth was considered the statistical unit. Pre-test failures were considered as 0 MPa for statistical analyses.

2.5. *Dentin etching patterns*

Sixteen mid-coronal dentin discs measuring roughly 1 mm in thickness were sectioned from sound third molars using a slow speed diamond saw (Isomet, Buehler Ltd.). The absence of enamel remnants was verified with a stereomicroscope (Leica M60, Leica Microsystems) at 40× magnification. Uniform standardized smear layers were created on the occlusal surface by wet-polishing the exposed dentin surfaces for 30 s with 320-grit SiC paper (Buehler-MET II, Buehler). The occlusal surfaces were etched with H_3PO_4 for 0 s (self-etch mode), 3 s, or 15 s, rinsed for 15 s, blot dried, the universal adhesive was actively applied for 20 s as previously described but left unpolymerized. Resin monomers were then copiously rinsed away with water spray for 15 s followed by ultrasonic agitation in distilled water for 5 min. Specimen were dehydrated in ascending ethanol series (50, 70, 80, 90 and 3 × 100%) and fixed in hexamethyldisilazane. Half of the specimens were longitudinally fractured in liquid nitrogen to expose a cross-sectional view of the etched surfaces, while the remaining specimens exposed an occlusal view of the dentin etching patterns. Samples were mounted on stubs, sputtered with gold/palladium and analyzed on backscattering mode at 10 kV (Phenom ProX, Phenom-World) at 1000× to 20,000× magnification.

2.6. *Hybrid layer morphology*

Two central resin-dentin beams from each tooth were randomly selected for hybrid layer evaluation under SEM. Beams were embedded in epoxy resin and wet-polished with 600-, 1200-, 2000- and 4000-grit SiC paper and 1, 0.25 and 0.05 μm polishing pastes (Buehler Ltd., Lake Bluff, IL, USA). Specimens were ultrasonically cleaned in distilled water after each polishing step for 5 min. Bonded interfaces were then treated with 50% H_3PO_4 for 5 s and 3% NaOCl for 10 min followed by dehydration in ascending ethanol series (50, 70, 80, 90 and 3 × 100%), fixed in hexamethyldisilazane, sputtered with gold/palladium and analyzed on backscattering mode at 10 kV (Phenom ProX, Phenom-World). A series of sequential micrographs of the entire bonded interfaces (2000× magnification) were obtained from each resin-dentin beam. Three randomly selected areas on each micrograph, located between adjacent resin tags, were analyzed by a single-blinded experienced examiner for hybrid layer thickness using an open-source image software (ImageJ, National Institute of Health, Bethesda, MD, USA). Measurements obtained from both beams were averaged and corresponded for the hybrid layer thickness of each specific tooth (n = 10).

2.7. *Statistical analyses*

Data normality and equality of variance were confirmed by the Shapiro–Wilk and Levene tests, respectively. 4-point

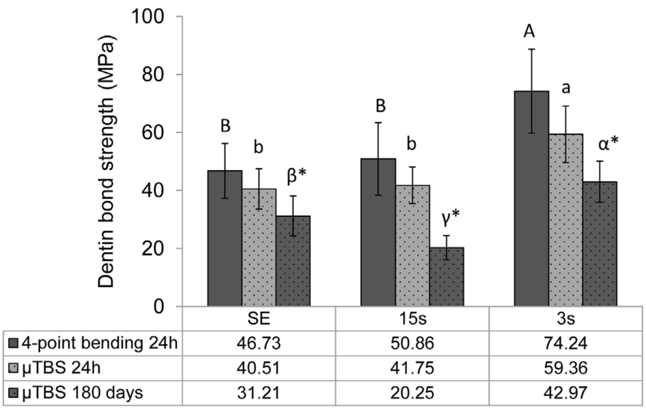

Fig. 2 – Quasi-static loading measurements (MPa) for the 4-point bending test at 24 h (n = 22) and the microtensile bond strength test (n = 10) at 24 h and after ageing in artificial saliva for 180 days at 37 °C. Resin-dentin beams were bonded to dentin using Scotchbond Universal (3M ESPE) in self-etch and etch-and-rinse modes: the latter consisted of H_3PO_4-etching times of 3 and 15 s. Columns identified by different capital letters represent significant differences according to Tukey's test (*p* < 0.05) between the 4-point bending groups. Columns identified by different lowercase letters represent significant differences between the microtensile groups tested at 24 h. Greek letters indicate significant differences between microtensile groups tested after ageing. * indicates significant reductions in bond strength of aged microtensile groups compared to those tested at 24 h within application modes.

flexure strengths obtained after quasi-static loading measurements were analyzed using a one-way ANOVA and Tukey test. Microtensile data was analyzed by two-way ANOVA and Tukey test. Fatigue life distributions were compared using the Wilcoxon Rank Sum Test. Hybrid layer thickness was analyzed by the Kruskal–Wallis test. Significance levels were set at 5% (α = 0.05). Statistical analyzes were performed on IBM SPSS Statistics for Windows, version 23 (IBM Corp., Armonk, NY, USA).

3. Results

3.1. *Quasi-static 4-point flexural strength*

One-way ANOVA revealed that dentin-etching times had a significant effect ($p < 0.001$; $\eta^2 = 0.503$) on the 4-point flexural strength of the tested universal adhesive. Bond strengths are reported in Fig. 2. TBI specimens (overall mean cross-sectional and standard deviation: 0.83 mm^2 ± 0.14) presented no significant differences regarding specimen size between groups (p = 0.527). No significant differences were detected between self-etch mode and dentin-etching for 15 s. Dentin-etching for 3 s produced significantly higher flexural strengths in the order of 59% and 46% compared to the self-etch mode and 15 s etching, respectively. Fractured surfaces were examined to identify the origins of failure and characteristics of the interface. Representative fracture patterns are shown in Fig. 3A. All fractures involved the bonded interface without any pure cohesive fractures in dentin or composite. The formation of compression shear lips indicates the initiation of failure on the tensile side for all specimens. Specimens bonded in self-etch mode presented higher surface areas characterized as adhesive failures at/or immediately below the hybrid layer exposing the underlying dentin surface. Dentin beams etched for 3 or 15 s were characterized by a discreet increase in adhesive failures above the hybrid layer.

Table 1 – Stress-life fatigue response, power law constants and estimated endurance limits for SU resin-dentin interfaces with different H_3PO_4-etching times.

	A (MPa)	B	R^2	Endurance limit (MPa)
Self-etch	68.211	−0.121	0.64	9.70
15 s	55.894	−0.065	0.71	19.60
3 s	64.492	−0.061	0.61	24.13

R^2 values represent the coefficient of determination for each model. Endurance limits were calculated at 1×10^7 cycles.

3.2. *Fatigue behavior and resistance*

Fatigue life diagrams for the TBI specimens are shown in Fig. 4. Basquin-type power law models are listed for all groups, which describe the mean fatigue strength distribution for each bonding protocol. According to the Wilcoxon Rank Sum test, all groups presented significant differences. The highest fatigue strength occurred after etching dentin for 3 s, followed by 15 s etching. The use of the universal adhesive in self-etch mode produced the lowest fatigue strengths. The stress-life fatigue constants for all groups are listed in Table 1 and were used to estimate the apparent endurance limit for each of the bonding protocols at 1×10^7 cycles. The endurance limits for 15 s and 3 s etching were 102% and 149% higher than the self-etch mode, respectively. Crack initiation sites varied according to bonding protocols (Fig. 3A; B). Composite cohesive cracks were frequently found between filler particles or even rupturing them in approximately 57% of analyzed surfaces. Crack distribution following type I, II, III and VI were fairly similar among all groups. Nonetheless, reduction in the number of cracks located exclusively at the dentin-adhesive layer (type IV) occurred after dentin etching for 3 or 15 s.

3.3. *Microtensile bond strength*

The mean cross-sectional area of tested resin-dentin beams 0.80 mm^2 (±0.22) ranged from 0.71 to 0.98 mm^2 without significant differences regarding specimen size between groups (p = 0.651). Two-way ANOVA revealed that dentin-etching times ($p < 0.001$; $\eta^2 = 0.622$), aging ($p < 0.001$; $\eta^2 = 0.58$) and their interactions ($p < 0.029$; $\eta^2 = 0.123$) had significant effects on microtensile bond strength of the tested universal adhesive. Table 2 shows bond strength means and the number of specimens following the fracture mode classification for all groups. Bond strengths are reported graphically in Fig. 2. At 24 h, significantly higher bond strength values, in the order of 47%, were obtained when dentin was etched for 3 s. No significant differences were detected between self-etch mode and

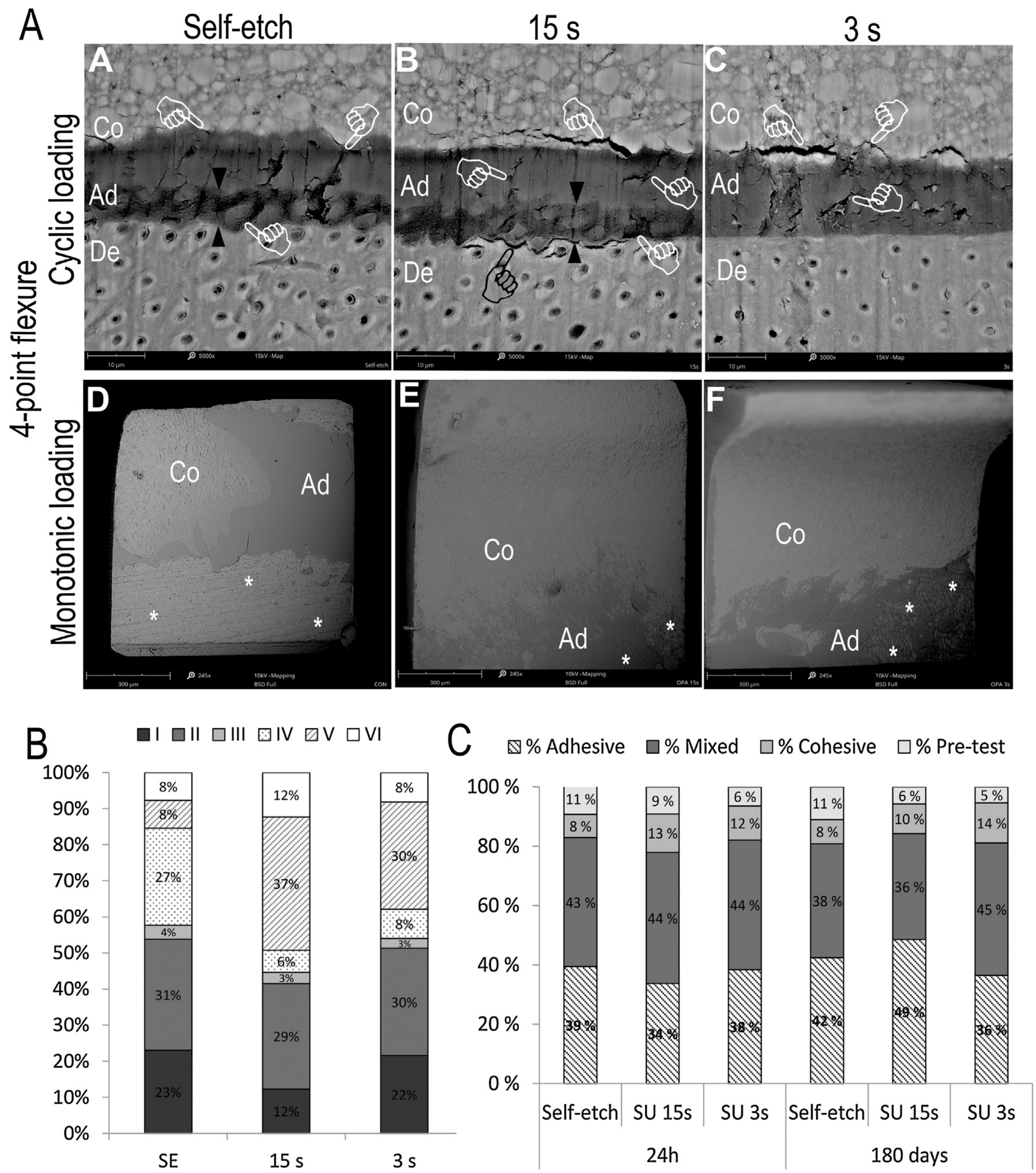

Fig. 3 – (A) Representative SEM micrographs of TBI resin-dentin specimens bonded with Scotchbond Universal (3M ESPE) in self-etch mode and after dentin-etching for 3 and 15 s. (A'-C') Profile view of unfractured specimens subjected to a minimum of 10^4 cycles. (D'-F') Fractured dentin surfaces of specimens tested under quasi-static monotonic loads. (Co = composite resin; Ad = adhesive layer; De = dentin). (B) Crack distribution patterns of TBI samples fatigued to a minimum of 10^4 loading cycles. (C) Failure mode distribution for microtensile samples after 24 h and 180 days of storage in artificial saliva.

15 s etching at 24 h. After the 6 month storage period, all groups presented significant reductions in microtensile bond strengths compared to 24 h groups. Etching for 3 s produced significantly higher bond strengths after aging compared to the self-etch mode in the order of 38% and the lowest bond strengths occurred when dentin was etched for 15 s. Cohesive fractures in dentin or composite ranged between 8 and 14% (Fig. 3C). The most common failure pattern was mixed with a substantial increase in the number of adhesive failures when dentin was etched for 15 s after 6 months.

3.4. *Dentin etching patterns*

SEM imaging (Fig. 5A–H) exhibited substantial morphological differences between etching times followed by the application of the universal adhesive: including different dissolution levels of smear layer and peritubular dentin, smear plug removal and overall exposure of collagen fibrils. Demineralized collagen was observed in all groups, with higher exposure for longer etching times. The self-etch application mode sparsely exposed collagen fibrils and remnant smear plugs

Table 2 – Microtensile bond strength means (MPa), standard deviations (± SD) and fracture modes.

	24 h	180 days
Self-etch	40.51 ± 6.96 [Ba] (30/33/6/8/77)	31.21 ± 6.87 [Bb] (31/28/6/8/73)
15 s	41.75 ± 6.31 [Ba] (26/34/10/7/77)	20.25 ± 4.17 [Cb] (34/25/7/4/70)
3 s	59.36 ± 9.73 [Aa] (30/34/9/5/78)	42.97 ± 7.12 [Ab] (27/33/10/4/74)

Tooth was considered the statistical unit (n = 10). Different capital letters indicate significant difference according to Tukey test ($p < 0.05$) when analyzed per row. Different lowercase letters indicate significant difference according to Tukey test ($p < 0.05$) when analyzed per column. Numbers in parentheses represent the total number of specimens following the fracture mode classification (1/2/3/4/5): 1) adhesive failure; 2) mixed failure, 3) cohesive failure, 4) pre-test failure and 5) total number of tested specimens.

were extensively identified. Peritubular cuffs presented no clear dissolution producing small-diameter-obliterated dentinal tubular orifices. Smear plugs could be identified deep into the dentinal tubules from a cross-sectional view. Dentin etching for 3 s followed by application of the universal adhesive completely dissolved smear plugs producing a thin collagen mesh with apparently smaller fibrillar diameter within intertubular dentin. Exposed collagen could not be identified inside dentinal tubules in areas composed by peritubular dentin. Etching for 15 s followed by adhesive application completely removed the smear layer exposing a thick shaggy-carpet like collagen mesh, extending inside the dentinal tubules (~10 μm).

3.5. *Hybrid layer morphology*

Kruskal–Wallis revealed that etching times significantly affected hybrid layer thickness ($p < 0.001$; $\eta^2 = 0.873$). Mean hybrid layer thickness and standard deviations for the etching times of 15 s, 3 s and the self-etch mode are 4.38 (±0.72), 2.83 (±0.48) 0.59 (±0.10) μm, respectively. All groups presented significant differences. Representative SEM images are shown in Fig. 5(I–K).

4. Discussion

H_3PO_4-application times resulted in different extensions of dentin demineralization and substantial changes in the fatigue behavior of resin-dentin interfaces bonded with the tested mild universal adhesive. This lead to the rejection of the first null hypothesis. Dentin-etching had significant effects on the dentin bonding performance of the tested universal adhesive. This suggests that the application mode for universal adhesives cannot be sidelined to create a hybridized dentin complex with adequate physical integrity and strength. Hence, this study produced compelling evidence that the selection between self-etch and etch-and-rinse application modes cannot be just a simple matter of user preference due to differences in the strength of bonded interfaces.

Since the groundbreaking introduction of the microtensile test in 1994 [26], resin-dentin bond strength assessment by "micro" quasi-static loading tests gained popularity in the dental science community. The staggering publication of 100-plus papers per year in the past decade reflects the wide acceptance and advantages of microtensile testing for resin-dentin bonding assessment including among others: (i) calculation of bond strength means and variances for single teeth; (ii) higher adhesive failures and fewer cohesive failures than previously used "macro" tests; (iii) measurement of higher interfacial bond strengths testing of small areas (*i.e.* < 1 mm^2) facilitates subsequent SEM/TEM examinations of the failed interfaces; (iv) regional measurements; (v) relatively easy and inexpensive test setup compared to more complex and time-demanding approaches (*e.g.* interfacial fracture toughness; strain energy release rates) [21,27–29]. The acceptance that μ*TBS* testing has adequate discriminative power [27,30] as well as a fairly well correlation to clinical outcomes [31] characterize it as a good predictor of resin-dentin bonding performance.

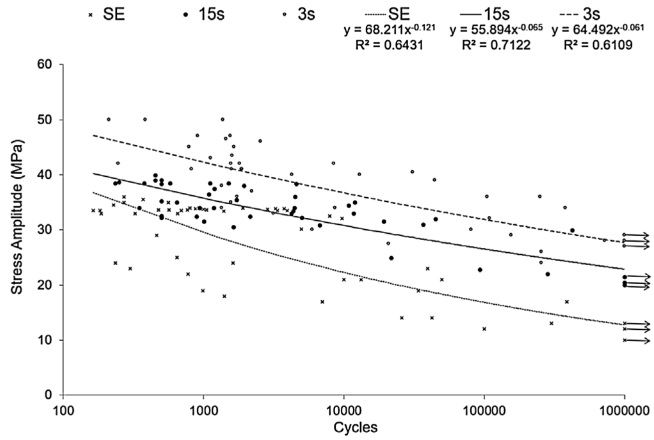

Fig. 4 – Stress life diagrams for the resin-dentin bonded specimens in self-etch mode and after dentin-etching for 3 and 15 s. Note that data points with arrows represent those specimens that reached 1.2 × 10^6 cycles and the test was discontinued. The R^2 values represent the coefficient of determination.

Bond strengths are dependent on flaws existing within or between materials, specimen size and geometry, material properties of each component of the adhesive joint, testing method and type of load application among others [27,32]. In the present study, resin-dentin bonding performance was assessed by bond strength tests under different conditions (*i.e.* microtensile at 24 h, 180 days and 4-point bending) and a modified "micro" TBI approach for fatigue testing. Cross-sectional bonding areas and crosshead speeds were kept constant for all test setups. Bond strength outcomes produced at 24 h were similar for μ*TBS* and 4-point bending regarding between-groups comparisons (*i.e.* 3 s > SE = 15 s for both methods). Not surprisingly, 4-point bending produced nominal bond strengths roughly 20% higher than the μ*TBS* values. This is related to the intrinsic characteristics of flexural tests which tend to produce higher values compared to tensile tests for the same material [33,34] and resin-dentin bonded interfaces [35]. A higher discrepancy between tensile and flexural strengths has been reported [35]. This can be attributed to differences in

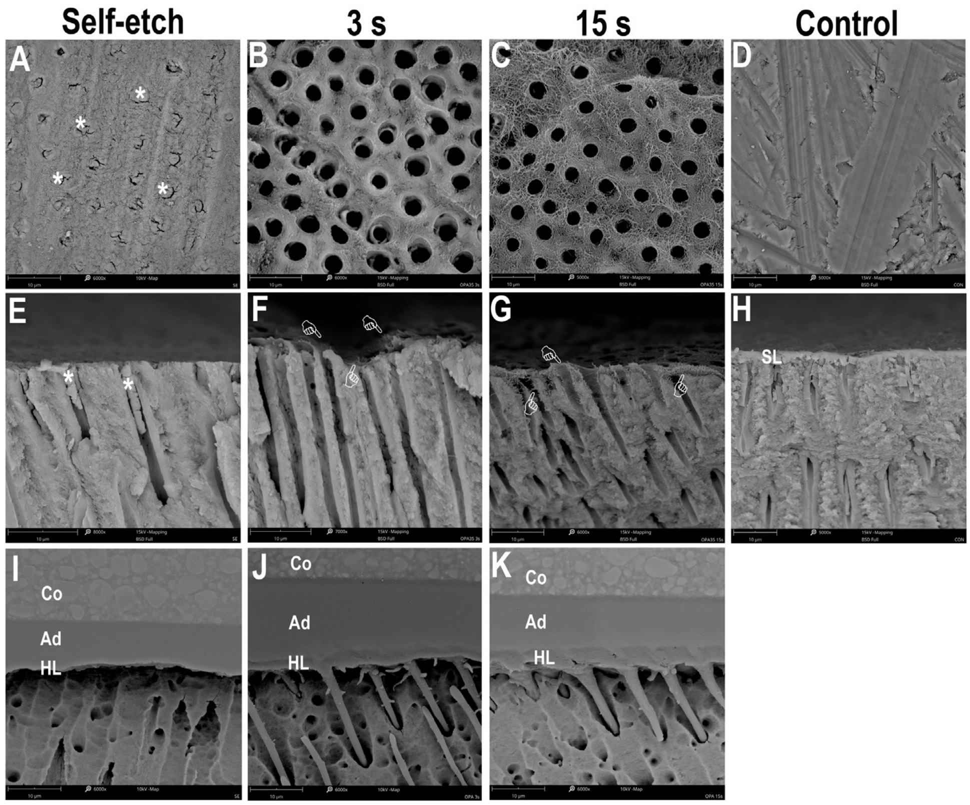

Fig. 5 – Representative SEM micrographs showing occlusal (A—C) and cross-section (E—G) views of distinct dentin etching patterns for Scotchbond Universal (3M ESPE) produced after self-etch application and in association with H_3PO_4-etching for 3 and 15 s. (D and H) representative images of loosely attached smear layers to the underlying dentin produced by 320-grit SiC paper. (A and E) Note the reduction of smear layer residues with longer H_3PO_4 etching times and the inability of the mild adhesive to properly etch through its entire extension. (I) hybrid layers produced in self-etch mode (0.59 ± 0.10 μm) were mostly absent of resin tags. Identifiable resin tags were irregular and <1.5 μm in length. (J) 3 s dentin etching produced hybrid layers 2.83 ± 0.48 μm thick. Resin tags were mostly cylindrical, well-formed, with uniform distribution and presented smooth superficial texture with extensions into the lateral branches of the tubules. (K) conventional 15 s dentin etching produced thicker hybrid layers (4.38 ± 0.72 μm) with consistent distribution of long funnel-shaped resin tags. Lateral extensions were also identified. * indicates smear plugs and white pointing fingers show demineralized collagen fibrils. (SL = smear layer; Co = composite; Ad = adhesive; HL = hybrid layer).

the flexural test setups: 4-point bending in the present study *vs* 3-point bending in [35]. Four-point bending setups generally require lower loadings forces for failure due to the larger portions of the specimen submitted to stress compared to 3-point bending. In the latter, stresses are mostly concentrated in a narrow volume under the loading nose, while 4-point bending presents a more uniform stress distribution in a larger volume between the two loading noses. According to the Griffith Theory, this increases the likelihood of submitting a greater number of flaws to stresses and thus crack formation, even at lower loads.

As commonly seeing in flexural setups, in 4-point bending compression and tensile stresses are produced on opposing sides of the beam with the highest stresses localized on its outer most portions, respectively [17]. Since brittle materials generally fail under tensile stresses before failing as a result of compressive stresses, the maximum tensile stress value sustained before failure may be considered as flexural strength. In 4-point bending setups, little to no shear stresses are produced between the two loading noses conferring a more reliable mechanical strength testing from the perspective of tensile stresses. In theory, no differences in flexural and tensile strengths should exist in isotropic-flawless materials. Although the anisotropic nature of resin-dentin interfaces could challenge the former assertion, tensile stresses should still be considered the primary cause of failure of the TBI interfaces under 4-point bending. This becomes more evident considering the substantially larger tensile strain development within the hybrid layer and resin adhesive compared with surrounding areas where cracks initiate [17]. Furthermore, the invariable presence of flaws within bonded interfaces concentrates stresses locally producing localized weakened areas more prone to failure. In the microtensile setup, the existence of mainly tensile stresses along the whole extension of the specimens increases the likelihood of superficial and inner flaws to grow into cracks more promptly than under flexure loads. Under flexure, only a reduced portion of the specimen is exposed to maximum tensile stress, which reduces the formation of potential weakened areas. Interestingly, no pure cohesive fractures in either composite or dentin occurred during the 4-point flexure test; all failures involved the bonded interface. This has been documented previously in flexural tests of resin-dentin bonded interfaces [36]. Cohesive failures in dentin or resin composite preferably should not be considered statistically, as these data would not truly represent interfacial strengths [32]. Therefore, the monotonic 4-point bending test may be a suitable option to eliminate intercurrences related to non-interfacial fractures on bond strength testing.

While in both tensile and flexural setups, non-uniform stress distributions generally occur along specimens due to a number of geometrical, loading and material properties, flexural-tests concentrate tensile stresses mostly on one side of the bonded interface [16]. This originates and guides crack progression throughout the weaker adhesive interface [36]. The relatively high number of cohesive fractures observed for the microtensile testing and the higher discriminative power of the cyclic loading approach test reopens the question of whether quasi-static bond strength testing is the most adequate approach to assess resin-dentin bonding reliability. It is important to note that strength-based testing is not an inherent material property of the bonding capacity of restorative materials to tooth structure [27]. Flaws produced during bonding may be considered weak links depending on their extension. Cyclic loading enables them to grow and become critical based on their location, loading forces and stress state. The major concern with quasi-static loading is that initially uncritical flaws are ignored, yet they may eventually become critical with clinical function and material degradation over time. Therefore, flaw distribution within the bonded interfaces plays a crucial role on bonding performance [16,27] and its impact may be underestimated by conventional quasi-static bond strength testing.

Few investigations have dealt with the fatigue strength of resin-dentin interfaces until now [17,25,35–37]. Fatigue can be defined as the reduction in load-bearing capacity of a material or interface subjected to cyclic stresses resulting from accumulation and coalescence of flaws leading to crack formation and thus fracture. Most importantly, fatigue failures occur at stresses that are generally much lower than the static “strengths” measured by microtensile or other monotonic tests [16]. The high stresses produced by quasi-static monotonic tests are hardly achieved during normal mastication. Hence, acknowledging only monotonic values may lead to false implications regarding resin-dentin bonding performance for actual clinical failures usually happen before reaching high stresses under cyclic loading. As such, the number of cycles to failure is largely a function of the defect population and size, rather than simply maximum stress. Previous publications have not reported significant differences between immediate bond strengths of universal adhesives used in self-etch or conventional etch-and-rinse mode [4,6,7], which is in accordance with our 24 h microtensile and 4-point flexural findings. However, significant differences in fatigue strength were found (*i.e.* 3 s>15 s > SE) according to the Wilcoxon Rank Sum test. In fact, the same bonding protocols presenting similar microtensile and 4-point flexural bond strengths at 24 h (*e.g.* SE and 15 s) produced substantially different fatigue resistances. Since fatigue testing proved to be more discriminative than conventional microtensile testing, the second null hypothesis was rejected. Basquin-type power law models were used to outline the mean of each fatigue strength distribution and subsequent estimation of the endurance limit for each bonding protocol. The apparent endurance limit of dentin surfaces H_3PO_4-etched for 15 s (19.6 MPa) was two-fold higher compared to the self-etch mode (9.7 MPa), while 3 s etching produced an even greater 2.5-fold increase (24.1 MPa). The lower slope observed in the fatigue life diagram (Fig. 4) for the self-etch mode indicate reduced reliability under cyclic loading compared to etch-and-rinse bonded interfaces. Although the apparent endurance limit of dentin etched for 3 s was roughly 20% higher than 15 s etching, Basquin exponents in Table 1 (identified by B) indicate no distinct trend in the reduction of fatigue life distributions of bonded interfaces etched for 3 or 15 s. This implies that the reduction in stress amplitude (MPa) values with increasing cycles is similar in both 3 s and 15 s etching. Therefore, the effect of cyclic mechanical degradation is similar on both bonding protocols. Although improvements in bonding performance were observed for the etch-and-rinse groups in comparison to the self-etching approach, longer H_3PO_4 application times do not seem to further benefit fatigue strength. Clearly, such findings must not be underestimated during clinical decision making. Since no significant differences were observed for both etching times, less aggressive etching approaches should be recommended to prevent overexposure of demineralized collagen fibrils and thus delay long-term degradation [4].

By examining the dentin-etching patterns and hybrid layer morphology of the tested bonded interfaces, it becomes evident that the tested mild universal adhesive is limited to etch through smear layers produced by the 320-grit SiC paper, as previously reported [4]. A similar issue has been reported for mild self-etch adhesives when thicker/denser smear layers were present [38]. Although SiC-papers do not exactly mimic bur-produced smear layers [39,40], those produced by 320-grit SiC paper tend to be more clinically relevant than those produced by 600-grit papers [40]. The general consensus that the resin-dentin bonding efficiency of universal adhesives does not depend on the application mode [6,10–12] was challenged by this study. Failure to produce more relevant smear layers and/or the use of quasi-static loading methods, with lower discriminative power, may explain the inability of previous studies to identify the reduced bonding performance of universal adhesives in self-etch mode [6,10–12]. Unetched smear layer residues could be observed after the application of the universal adhesive in self-etch for 20 s following manufactures' recommendations (Fig. 5A). Dentin etching for 15 s, removed the entire extension of the smear layer exposing a great number of demineralized collagen fibrils (Fig. 5C). Similarly, 3 s H_3PO_4-etching removed the majority of the smear layer; however, with considerably lower collagen exposure (Fig. 5B). As a consequence, 15 s etching produced significantly thicker hybrid layers, followed by 3 s etching and the self-etch mode application. Inefficient interaction with the underlying dentin [4] creates extensive adhesive-smear-only bonded areas which may indeed be considered as resin-dentin bonding flaws, albeit they are not necessarily characterized as visible empty spaces. In such interfaces, interaction between adhesive and dentin is intermingled with weakly-bonded adhesive-smear bundles increasing flaw distribution when compared to etched samples. Considering that defects as small as a few micrometers in size may affect the fatigue strength of brittle materials [41], incorporation of such discontinuities within self-etched bonded interfaces certainly contributed to substantially lower fatigue strengths. This can also be confirmed by the crack initiation site analyses (Fig. 3B). One major advantage of using the TBI fatigue approach is that unfractured bonded interfaces can be used to identify zones more prone to crack initiation. Since samples submitted to higher cyclic loading (*i.e.* $>10^4$ cycles) were included in this analysis, it is only natural that cracks could be invariably identified at all material junctions (Fig. 3A). This reflects the strenuous effect of fatigue over brittle interfaces. While samples bonded without etching presented a higher incidence of crack initiation located exclusively at the adhesive-dentin interface, improving the adhesive-dentin interaction by etching shifted crack formation towards the adhesive-composite interface. The added benefits of smear layer removal without overexposure of demineralized collage, produced by 3 s H_3PO_4-etching, contributed to a more favorable bonding substrate with preserved hydroxyapatite. This certainly improved the chemical bonding between 10-MDP acidic monomers and dentin resulting in the most effective bonding protocol in this study. While in self-etch mode smear layer prevented optimum interaction between the adhesive and the underlying dentin, in conventional 15 s H_3PO_4-etching chemical bonding was likely inexistent.

Unfortunately, there are many more threats to resin-dentin bonding [1,42,43] than just mechanical fatigue as a result of cyclic loading. Although the fatigue approach proved to be more discriminative than the conventional monotonic testing to determine the bonding efficiency of resin-dentin bonded interfaces, the cyclic loading approach applied in this study does not take into consideration the effect of hydrolysis of both resin and organic components present at the bonded interface over time. Samples submitted to the longest loading cycles were in water at most for a few days during testing. In order to replicate the detrimental effects of hydrolysis, longer storage times are required. The superior long-term bond strengths of universal adhesives in self-etch mode compared to 15 s etching agrees with previous studies [4,7]; however, such implication must be analyzed with caution. It is unquestionable that the rapid degradation of collagen fibrils by endogenous enzymes deeply impairs resin-dentin bonding over time [42–44]. Since self-etch bonding exposes considerably less collagen fibrils, it is reasonable to assume that the negative impact of collagen hydrolysis over time in resin-dentin bonding would be diminished. This could provide valid arguments to support the use of universal adhesives in self-etch over etch-and-rinse mode [8–10]. However, the devastating effects of cyclic loading must also be considered for an evidence-based decision making. Future studies assessing the effect of long-term storage and cyclic loading on the stability of resin-dentin interfaces created by different universal adhesives are still needed. The data produced by the different test methods employed here produced compelling evidence that applying universal adhesives in self-etch mode on clinically relevant smear layers can produce disappointing dentin-bonding outcomes. In order to obtain the most favorable bonding protocol, a separate etching step capable of removing the smear layer without overexposing collagen fibrils seems necessary for mild universal adhesives.

5. Conclusion

The immediate quasi-static loading microtensile and 4-point flexural tests were limited to identify differences in resin-dentin bonding performance between application modes for the tested universal adhesive. Fatigue testing proved to be a more discriminative test revealing a suboptimal bonding performance of dentin-composite interfaces created with mild universal adhesives in self-etch mode. Smear layer removal with phosphoric acid substantially improved the endurance limits for such bonded interfaces, especially for shorter dentin-etching times. Conventional H_3PO_4 dentin-etching improved the fatigue strength; however, such benefits may be overshadowed by the overexposure of collagen fibrils and subsequent endogenous proteolytic hydrolysis on the long-term. Hence, the answer to the clinical dilemma of whether to etch or not to etch dentin, considering solely the adhesive interface quality of universal adhesives, may reside on a less-aggressive separate etching approach.

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